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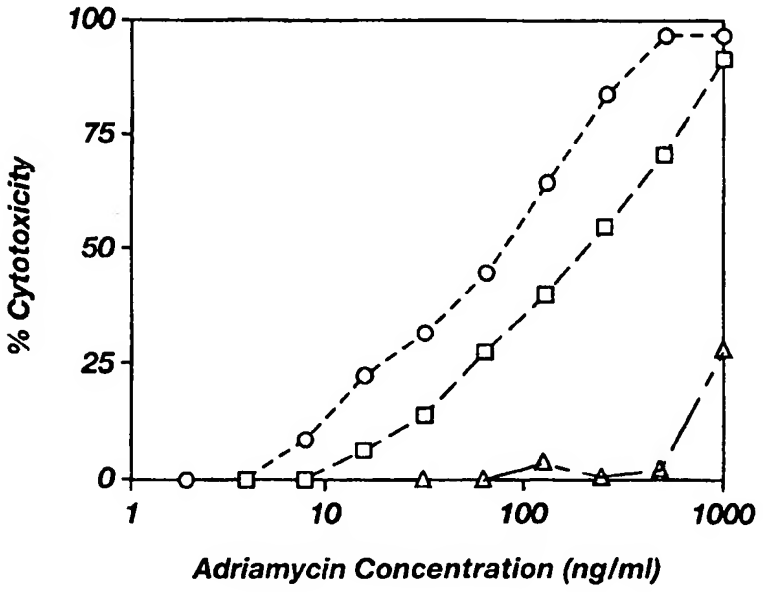
(54) Title: CONJUGATES TARGETED TO THE TARGET RECEPTORS AND/OR INTERLEUKIN-2 RECEPTORS

(57) Abstract

A composition for intracellular delivery of a chemical agent into a target receptor and/or interleukin-2-receptor-bearing cell, e.g. an activated T cell and cancer cell, includes a chemical agent, at least one copy of target-receptor binding and/or an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a target receptor and/or IL-2 receptor on the target receptor and/or IL-2-receptor-bearing cell and elicits endocytosis of the composition. The composition also optionally includes a biodegradable spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivatives thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compositions for delivering a chemical agent *in vivo* or *in vitro* are also disclosed. A method of detecting a disease, such as cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma, associated with elevated levels of soluble target receptor and/or IL-2 receptor is also disclosed.



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CONJUGATES TARGETED TO THE TARGET RECEPTORS AND/OR INTERLEUKIN-2 RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 08/914,042, filed August 5, 1997.

BACKGROUND OF THE INVENTION

This invention relates to delivery of chemical agents to cells. More particularly, this invention relates to compositions and methods for intracellular delivery of chemical agents to a specific cell type, i.e. cancer cells and /or cells bearing the interleukin-2 (IL-2) receptor.

Toxins that target cell surface receptors or antigens on tumor cells have attracted considerable attention for treatment of cancer. E.g., I. Pastan & D. FitzGerald, Recombinant Toxins for Cancer Treatment, 254 Science 1173 (1991); Anderson et al., U.S. Patent Nos. 5,169,933 and 5,135,736; Thorpe et al., U.S. Patent No. 5,165,923; Jansen et al., U.S. Patent No. 4,906,469; Frankel, U.S. Patent No. 4,962,188; Uhr et al., U.S. Patent No. 4,792,447; Masuho et al., U.S. Patent Nos. 4,450,154 and 4,350,626. These agents include a cell-targeting moiety, such as a growth factor or an antigen-binding protein, linked to a plant or bacterial toxin. They kill cells by mechanisms different from conventional chemotherapy, thus potentially reducing or eliminating cross resistance to conventional chemotherapeutic agents.

Copending PCT Patent Application Serial No. PCT/US95/11515 (WO 96/08263) filed September 12, 1995, describes compositions and methods for specific intracellular delivery of a chemical agent into a CR2-receptor-bearing cell, *e.g.* B lymphocytes. The compositions comprise a CR2-receptor-binding and endocytosis-inducing ligand (CBEL) coupled to the chemical agent. The CBEL binds to the CR2 receptor on the surface of B lymphocytes and elicits endocytosis of the composition such that the composition is transported to lysosomes. In the lysosomes, the chemical agent is preferably separated from the remainder of the

composition such that the chemical agent can be transported or diffuse into the cytoplasm or nucleus. Optionally, the composition can include a spacer, which can be either biodegradable (in the lysosome) or non-biodegradable, for coupling the CBEL to the chemical agent. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate.

Copending PCT Patent Applications, Serial No. PCT/US97/03832, filed March 12, 1997, and Serial No. PCT/US98/09057, filed May 4, 19987, describe compositions and methods for specific intracellular delivery of a chemical agent into T lymphocytes. The compositions are represented by the formula $[L\text{-}S]_a\text{-}C\text{-}[S\text{-}A]_b$ wherein L is a ligand configured for binding to a receptor on a T lymphocyte and stimulating receptor-mediated endocytosis of the composition, A is a chemical agent, S is a spacer moiety, C is a water soluble polymer having functional groups compatible with forming covalent bonds with the ligand, chemical agent, and spacer, and a and b are positive integers. These compositions are also designed to be transported to lysosomes, where the chemical agent is separated from the remainder of the composition for diffusion or transport to other locations in the cell. Preferred water soluble polymers include poly(ethylene glycol) and a copolymer of N-(2-hydroxypropyl)methacrylamide (HPMA). Preferred chemical agents include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. The composition can further comprise a carrier such as other water soluble polymers, liposomes, or particulates.

It would also be advantageous to develop additional compositions that are specifically targeted to other receptors on T lymphocytes or cancer cells. For example, targeting of T lymphocytes would enable therapeutic applications for T-cell-associated diseases and tissue graft rejection. Such T-cell-associated diseases include arthritis, T-cell lymphoma, skin cancers, psoriasis, and diseases resulting from HIV infection.

In view of the foregoing, it will be appreciated that compositions for intracellular delivery of chemical agents to cancer cells and/or T cells and methods of use thereof would be significant advancements in the art.

BRIEF SUMMARY OF THE INVENTION

It is an object of the present invention to provide compositions for intracellular delivery of selected chemical agents to a specific cell type, *i.e.* Cancer cells or IL-2-receptor-bearing cells.

It is also an object of the invention to provide methods of making and methods of using compositions for intracellular delivery of selected chemical agents to cancer cells and/or IL-2-receptor-bearing cells.

It is another object of the invention to provide compositions and methods for delivering selected chemical agents to cancer cells and/or IL-2-receptor-bearing cells using water soluble polymers that are inexpensive, biocompatible, and resistant to development of an antibody response.

It is still another object of the invention to provide compositions and methods of use thereof for intracellular delivery of selected chemical agents to cancer cells.

It is yet another object of the invention to provide compositions and methods of use thereof for intracellular delivery of selected chemical agents to activated T cells.

It is yet another object of the invention to provide a conjugate composition of a peptide and a pendent PEG and the equivalent thereof and a method of making thereof.

These and other objects are achieved by providing a composition for intracellular delivery of a chemical agent into a targeted cell type such as an IL-2-receptor bearing cell, the composition comprising (a) a water-soluble, biocompatible polymer, (b) the chemical agent covalently, releasably coupled to the polymer, and (c) a ligand comprising an targeted receptor-binding peptide covalently coupled to the polymer. In a preferred embodiment of the invention, the composition further comprises a biodegradable peptide.

Preferably, the biocompatible polymer is a polyalkylene oxide. Preferred polyalkylene oxides are selected from the group consisting of alpha-substituted polyalkylene oxide derivatives, polyethylene glycol homopolymers and derivatives thereof, polypropylene glycol homopolymers and derivatives thereof, alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), branched polyethylebe glycols, star polyethylene glycols, pendent polyethylebe glycols, block copolymers of poly(alkylene oxides) and the activated derivatives thereof. Preferably, the polyalkylene oxide has a molecular weight of about 200 to about 50,000. More preferably, the polyalkylene oxide has a molecular weight of about 2,000 to about 20,000. Most preferably, the polyalkylene oxide has a molecular weight of about 5,000. Especially preferred polyalkylene oxides are polyethylene glycol and polyethylene oxide.

The target receptor-binding peptide is preferably a member selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof. More preferably, the target-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, and SEQ ID NO:22 through SEQ ID NO:47. Most preferably, the target-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:22, SEQ ID NO:31, and SEQ ID NO:46.

The chemical agent is preferably selected from the group consisting of cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

Preferably, the target binding peptide comprises a biodegradable portion, and a preferred biodegradable portion comprises Gly-Phe-Leu-Gly (SEQ ID NO:21).

In one preferred embodiment, the composition further comprises a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates. Preferably, such water soluble polymers are selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid), and N-substituted methacrylamide-containing polymers.

A method of delivering a chemical agent *in vitro* into an IL-2-receptor-bearing cell, or a cancer cell bearing the targeting receptor, in a population of cells, comprises the steps of:

(a) providing a composition comprising (i) a water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the polymer, and (iii) a ligand comprising an target-receptor-binding peptide covalently coupled to the polymer; and

(b) contacting the population of cells with an effective amount of the composition under conditions wherein the ligand binds to an target receptor on the target-receptor-bearing cell and elicits endocytosis of the composition.

A method of delivering a chemical agent into an target-receptor-bearing cell in a warm-blooded animal, comprises the steps of:

(a) providing a composition comprising (i) a water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the polymer, and (iii) a ligand comprising an target-receptor-binding peptide covalently coupled to the polymer; and

(b) administering to the warm-blooded animal an effective amount of the composition under conditions wherein the ligand contacts and binds to an target receptor on the target-receptor-bearing cell and elicits endocytosis of the composition.

The composition of the present invention can be administered either systemically or locally, depending on the individual's need.

Another aspect of the invention relates to a composition comprising a peptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47, amides and chemically modified equivalents thereof.

A method for detecting a disease associated with elevated levels of soluble target receptor in circulation comprising the steps of:

(a) providing a composition comprising a target receptor binding peptide;

(b) mixing the composition with a body fluid to be tested under conditions suitable for binding of the composition to said soluble target receptor in the body fluid to form a complex; and

(c) detecting the complex and determining whether the complex is present at elevated levels as compared to normal individuals.

Diseases that can be detected according to this method include cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma. "Body fluid", refers to any secretion or liquid composition carried on a warm blooded animal, such as blood serum, sweat, saliva, tear, urea, etc. Preferably, the body fluid that is tested is serum. Detection of the complex of peptide and soluble target receptor, i.e. interleukin-2 receptor, preferably comprises an enzymatic or radioactive-label sorbent assay.

Target receptor binding peptides that are suitable for this invention include SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47, amides or other chemical modifications that result in biologically functional equivalents thereof. Especially preferred peptides are SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:46, amides and other biologically functional equivalents thereof. As is well known in the art, such an amide is generally formed by reaction of an acid chloride of the peptide with ammonia, resulting in replacement of -OH group of the C-terminal carboxylic acid with $-NH_2$.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 shows the *in vitro* cytotoxic activity of a composition according to the present invention and control compositions against mouse CTLL-2 T cells: (□) PEG-TT23-ADR (SEQ ID NO:22); (△) PEG-GFLG-ADR (SEQ ID NO:21); and (○) unconjugated adriamycin.

FIG. 2 shows the *in vitro* inhibition of IL-2-induced proliferation of murine splenocytes using (□) PEG-TT23 (SEQ ID NO:22) and (△) unconjugated TT23 (SEQ ID NO:22).

FIG. 3 shows the *in vitro* cytotoxic dose response curves of a composition according to the present invention(Doxorubicin conjugated 20KD PEG-8PA-TT45(SEQ ID NO:31) against human cells: (○) Human B-cell lymphoma cell line: Raji(IC50=1.19mcM).; (△) Human T-cell lymphoma cell line:

HuT78(IC50=7.80mcM).; (□)Human B-cell lymphoma cell line: Daudi(IC50=2.08mcM).

FIG. 4 shows the *in vitro* cytotoxic activity of unconjugated Doxorubicin against human cells: (○) Human T-cell lymphoma cell line: HuT78(IC50=4.36mcM).; (△) Human B-cell lymphoma cell line: Daudi(IC50=0.19mcM).; (□)Human B-cell lymphoma cell line: Raji(IC50=0.18mcM).

DETAILED DESCRIPTION

Before the present compositions and methods for targeted delivery to target-receptor-bearing cells are disclosed and described, it is to be understood that this invention is not limited to the particular embodiments, process steps, and materials disclosed herein as such embodiments, process steps, and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a composition containing "a ligand" includes reference to two or more ligands, reference to "a chemical agent" includes reference to one or more of such chemical agents that may be the same or different chemical agents, and reference to "a spacer" includes reference to two or more spacers.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

As used herein, "peptide" means peptides of any length and includes proteins. The terms "polypeptide" and "oligopeptide" are used herein without any particular intended size limitation, unless a particular size is otherwise stated.

As used herein, "target-receptor-binding peptide", "IL-2-receptor-binding peptide" means a peptide configured for binding to an target receptor or IL-2

receptor and stimulating internalization thereof by receptor-mediated endocytosis. The target receptor ir preferably a receptor which is specifically or dominantly expressed in a cancer cell, it can be a receptor other an IL-2 receptor, or is a IL-2 receptor. According to the present invention, ligands comprising such target-receptor binding peptides or IL-2-receptor-binding peptides are coupled to various functional molecules so that upon endocytosis of the ligands the various functional molecules coupled thereto are also internalized by the cells.

Preferred target-receptor-binding peptides include the peptide having the amino acid sequence identified as SEQ ID NO:1 and biologically functional equivalents thereof. Such functional equivalents retain functionality in binding the target receptor or IL-2 receptor and eliciting receptor-mediated endocytosis although they may be truncations, deletion variants, chemically modified, substitution variants of SEQ ID NO:1 or include additional amino acid residues attached thereto. It is also preferred that the target-receptor-binding peptides have a size of about 6-20 amino acid residues, more preferably about 6-12 amino acid residues, and most preferably about 6-8 amino acid residues. More preferred target receptor binding peptides include SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47 and amides thereof. Especially preferred peptides are SEQ ID NO:22, SEQ ID NO:31 and SEQ ID NO:46, amides thereof and functional equivalents thereof.

As mentioned above, changes may be made in the structure of the target receptor-binding peptide while maintaining the desirable receptor-binding characteristics. For example, certain amino acid residues may be substituted for other amino acid residues in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites of ligands such as an IL-2 receptor-binding peptide. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the

sequence of an target receptor-binding peptide without appreciable loss of its biological utility or activity.

It is also well understood by the skilled artisan that inherent in the definition of a biologically functional equivalent protein or peptide is the concept that there is a limit to the number of changes that may be made within a defined portion of the molecule and still result in a molecule with an still remains acceptable level of equivalent biological activity. It is also well understood that where certain residues are shown to be particularly important to the biological or structural properties of a protein or peptide, e.g. residues in active sites, such residues may not generally be exchanged.

By "biologically functional equivalents" or "chemically modified equivalents", it means that one or more of the amino acids of the peptides of the present invention can be chemically modified, or substituted by its analogues without a significant loss of its target receptor binding activity. Various types of chemically modified amino acid analogues are commercially available and are well known to one skilled in the art.

Amino acid substitutions are generally based on the relative similarity of the amino acid side-chains relative to, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. An analysis of the size, shape, and type of the amino acid side-chains reveals, for example, that arginine, lysine, and histidine are all positively charged residues; that alanine, glycine, and serine are all a similar size; and that phenylalanine, tryptophan, and tyrosine all have a generally similar shape. Therefore, based upon these considerations, the following conservative substitution groups or biologically functional equivalents have been defined:(a) Cys; (b) Phe, Trp, Tyr; (c) Gln, Glu, Asn, Asp; (d) His, Lys, Arg; (e) Ala, Gly, Pro, Ser, Thr; and (f) Met, Ile, Leu, Val. M. Dayhoff et al., Atlas of Protein Sequence and Structure (Nat'l Biomed. Res. Found., Washington, D.C., 1978), hereby incorporated by reference.

To effect more quantitative changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, which are as follows:

isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art. J. Kyte & R. Doolittle, A simple method for displaying the hydropathic character of a protein, 157 J. Mol. Biol. 105-132 (1982), incorporated herein by reference. It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based on the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, within ± 1 is particularly preferred, and within ± 0.5 is even more particularly preferred.

It is also understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent protein. As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, within ± 1 is particularly preferred, and within ± 0.5 is even more particularly preferred.

A hexapeptide believed to be a part of IL-2 that binds to the IL-2 receptor has been identified (SEQ ID NO:1), D.A. Weigent et al., 139 Biochem. Biophys. Res. Commun. 367-74 (1986). Moreover, regions of homology between this IL-2 hexapeptide and env proteins of immunosuppressive retroviruses have been discovered. D.A. Weigent et al., *supra*; W.E. Reiher III et al., 83 Proc. Nat'l Acad. Sci. USA 9188-92 (1986). Thus, amino acid substitutions in these regions of

homology as compared to the IL-2 hexapeptide are also considered to be biologically functional equivalents. Therefore, illustrative biologically functional equivalents of SEQ ID NO:1 include the following: SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; and SEQ ID NO:6. Other illustrative biologically functional equivalents have also been discovered, including: SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11, SEQ ID NO:26, and SEQ ID NO:27. Additional biologically functional equivalents can be discovered by a person of ordinary skill in the art according to the guidance and principles disclosed herein without undue experimentation.

As used herein, "macromolecule" means a composition comprising a water soluble polymer with a ligand and a chemical agent releasably coupled thereto. Preferably the polymer is a polyalkylene oxide and the ligand is an oligopeptide. The chemical agent can be from many different classes of molecules, as explained in more detail herein.

As used herein, by "releasably coupled"or "releasable, covalent bond", it refers to the covalent bondings of the ligand, the chemical agent and the biocompatible polymer are biodegradable and the active agent releasable after the eliciting recepto-mediated endocytosis of the composition into the targeted cell.

As used herein, "prodrug" means a chemical agent that is chemically modified to overcome a biological barrier. When a chemical agent is converted into its prodrug form, its biological activity is eliminated or substantially reduced, but the biological barrier that inhibited its effectiveness is no longer problematic. The chemical group that is attached to the chemical agent to form the prodrug, *i.e.* the "pro-moiety", is removed from the prodrug by enzymatic or nonenzymatic means to release the active form of the chemical agent. See A. Albert, Chemical Aspects of Selective Toxicity, 182 Nature 421 (1958). The instant compositions are prodrugs because the chemical agent that has the selected effect when internalized in IL-2-receptor-bearing cells is modified with a ligand, water soluble polymer, and, optionally, spacers such that the composition is delivered into the target receptor and/or IL-2-receptor-bearing cells, thus penetrating the cell membrane thereof. The biological effect of the chemical agent is greatly reduced or eliminated until the

composition is delivered intracellularly and the chemical agent is released from the remainder of the composition by biodegradation of the spacer.

As used herein, "chemical agent" means and includes any substance that has a selected effect when internalized into a cancer cell and/or an IL-2-receptor-bearing cell. Certain chemical agents have a physiological effect, such as a cytotoxic effect or an effect on gene regulation, when internalized into the cell. A "transforming nucleic acid" (RNA or DNA), when internalized into a cell, can be replicated and/or expressed within the cell. Other nucleic acids can interact with regulatory sequences or regulatory factors within the cell to influence gene expression within the cell in a selected manner. A detectable "label" delivered intracellularly can permit identification of cells that have internalized the compositions of the present invention by detection of the label. "Drugs" or "pharmacologically active compounds" can be used to ameliorate pathogenic effects or other types of disorders. Particularly useful chemical agents include polypeptides, and some such chemical agents are active fragments of biologically active proteins, or are specific antigenic fragments (*e.g.*, epitopes) of antigenic proteins. Thus, chemical agents include cytotoxins, gene regulators, transforming nucleic acids, labels, antigens, drugs, and the like.

As used herein, "drug" or "pharmacologically active agent" means any chemical material or compound suitable for intracellular administration in a cancer cell or an IL-2 receptor bearing cell, e.g. an activated T lymphocyte, and /or a cancer, that stimulates a desired biological or pharmacological effect in such cell. Preferred drugs are cytotoxins and immunosuppressant drugs. Preferred cytotoxins are members selected from the group consisting of adriamycin, doxorubicin, taxol, cisplatin, methotrexate, cyclophosphamide and derivatives thereof. Preferred immunosuppressants are members selected from the group consisting of cyclosporin, rapamycin, FK506 and derivatives thereof. Other anticancer drugs are described in Cancer Medicine edited by James F. Holland et al., 1997, hereby incorporated by references.

As used herein, "carrier" means water soluble polymers, particulates, or liposomes to which a composition according to the instant invention can be coupled.

Such carriers increase the molecular size of the compositions and may provide added selectivity and/or stability. Such selectivity arises because carrier-containing compositions are too large to enter cells by passive diffusion, and thus are limited to entering cells through receptor-mediated endocytosis. The potential for use of such carriers for targeted drug delivery has been established. See, e.g., J. Kopecek, 5 Biomaterials 19 (1984); E. Schacht et al., Polysaccharides as Drug Carriers, in Controlled-Release Technology 188 (P.I. Lee & W.R. Good, eds., 1987); F. Hudecz et al., Carrier design: Cytotoxicity and Immunogenicity of Synthetic Branched Polypeptides with Poly(L-lysine) Backbone, 19 J. Controlled Release 231 (1992); Z. Brich et al., Preparation and Characterization of a Water Soluble Dextran Immunoconjugate of Doxorubicin and the Monoclonal Antibody (ABL364), 19 J. Controlled Release 245 (1992). Thus, illustrative water soluble polymers include dextran, inulin, poly(L-lysine) with modified epsilon-amino groups, poly(L-glutamic acid), N-substituted methacrylamide-containing synthetic polymers and copolymers, and the like.

As used herein, "effective amount" is an amount sufficient to produce a selected effect. For example, a selected effect of a composition containing a cytotoxin as the chemical agent could be to kill a selected proportion of IL-2-receptor-bearing cells, e.g. activated T cells, within a selected time period. An effective amount of the composition would be the amount that achieves this selected result, and such an amount could be determined as a matter of routine by a person skilled in the art.

The compositions of the present invention provide intracellular delivery of a chemical agent capable of eliciting a selected effect when delivered into an target-receptor-bearing cell. Illustrative embodiments of the composition comprises a ligand configured for binding to an target receptor or IL-2 receptor on the target-receptor-bearing cells and stimulating receptor-mediated endocytosis of the composition, a chemical agent and a water soluble polymer having functional groups compatible with forming a releasable, covalent bonds with the ligand. The binding ligand are peptides which are preferably comprises a biodegradable spacer such that the chemical agent is detached from the composition by hydrolysis and/or enzymatic

cleavage inside IL-2-receptor-bearing cells, e.g. Cancer cells or T cells, especially in lysosomes. Once detached, the chemical agent diffuses or is transported to other locations in the cell where it can exert its functional effect in the cell. Illustrative of such spacers is the peptide Gly-Phe-Leu-Gly (SEQ ID NO:21). Equivalent peptide spacers are well known in the art.

The water soluble polymer is preferably a poly(alkylene oxide). Within this group of substances are alpha-substituted polyalkylene oxide derivatives, such as methoxypolyethylene glycols or other suitable alkyl-substituted derivatives, such as those containing C_1-C_4 alkyl groups. Preferably the polymer is a monomethyl-substituted PEG homopolymer. Other poly(alkylene oxides) are also useful, including other polyethylene glycol (PEG) homopolymers and derivatives thereof, polypropylene glycol homopolymers and derivatives thereof, other alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), and block copolymers of poly(alkylene oxides) or activated derivatives thereof. Other preferred PEGs include branched, pendent and star PEGs, such as are commercially available from Shearwater Polymers, Inc. (Huntsville, AL). Y. Gnanou et al., 189 Makromol. Chem.2885(1988); D.Rein et al., 44 Acta Polymer. 225(1993); E.W. Merrill,U.S.Patent 5,171,264; Poly(Ethylene Glycol) Chemistry in Biotechnical and Biomedical Application , J.Milton Harris, 1992, hereby incorporated by reference. In those embodiments of the invention where PEG-based polymers are used, it is preferred that they have molecular weights of from about 200 to about 50,000. Molecular weights of about 2,000 to about 20,000 are preferred, and molecular weights of about 20,000 are particularly preferred. PEG is preferred because it is inexpensive, approved by the FDA for administration to humans, and is resistant to eliciting an antibody response. Poly(ethylene oxide) (PEO) is another preferred water soluble polymer represented by P. The coupling of a ligand to a chemical agent can be, without limitation, by covalent bond, electrostatic interaction, hydrophobic interaction, physical encapsulation, and the like. The most preferred polymer for the invention is a pendent PEG, or a star shaped PEG. It is another novel aspect of the present invention to provide a peptide

conjugated to a pendent PEG or a star shaped PEG and a method of preparing thereof.

The compositions of the present invention can further comprise a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates. Such water soluble polymers for use as carriers are selected from the group consisting of dextran, inulin, poly(L-lysine) (PLL) with modified epsilon amino groups, poly(L-glutamic acid) (PGA), N-substituted methacrylamide-containing polymers and copolymers, and the like. A preferred water soluble polymer is a copolymer of N-(2-hydroxypropyl)methacrylamide (HPMA).

Thus, according to the invention, the composition provides means for preferential binding to a target receptor such as IL-2 receptor, on activated T cells, thus triggering internalization of the composition by endocytosis. The chemical agent provides means for achieving a selected effect in the target-receptor bearing cells. Accordingly, for example, chemical agents comprise cytotoxins, including radionuclides, for selective killing or disabling of cells; nucleic acids for genetically transforming or regulating gene expression in cells; drugs or other pharmacologically active agents including immunosuppressant, for achieving a selected therapeutic effect; labels, including fluorescent, radioactive, and magnetic labels, for permitting detection of cells that have taken up the compositions; and the like.

IL-2 is a lymphocyte growth factor produced by T cells that is essential for a normal immune response. Binding of IL-2 to the IL-2 receptor precedes internalization by receptor-mediated endocytosis. The human IL-2 gene has been sequenced, T. Taniguchi et al., 302 Nature 305-10 (1983), hereby incorporated by reference, as has the gene for the human IL-2 receptor, W.J. Leonard et al., 311 Nature 626-31 (1984); T. Nikaido et al., 311 Nature 631-35 (1984); D. Cosman et al., 312 Nature 768-71 (1984). The IL-2 receptor is a heterotrimeric glycoprotein complex on the cell membrane with a 55 kDa α subunit, a 75 kDa β subunit, and a 64 kDa γ subunit. The only normal human tissues expressing the α and β subunits are activated T cells, B cells, LGL cells, and monocytes and some liver Kupffer cells, macrophages, and skin Langerhans' cells. A.E. Frankel et al., 11 Leukemia

22-30 (1997). A variety of hematologic neoplasms may show high affinity IL-2 receptor expression including hairy cell leukemia, adult T cell leukemia, and a fraction of cutaneous T cell lymphomas and B cell chronic lymphocytic leukemias. Recombinant toxins targeted to the IL-2 receptor have been described wherein the ligand is IL-2. A.E. Frankel et al., *supra*; U.S. Patent No. 4,675,382; J. vanderSpek et al., 268 J. Biol. Chem. 12077-82 (1993); I. Pastan & D. FitzGerald, *supra*.

In some embodiments of the present invention, the compositions are constructed by chemically conjugating the ligand and chemical agent to the water soluble polymer. "Chemically conjugating" the ligand and the chemical agent to the water soluble polymer, as that term is used herein, means covalently bonding the ligand and chemical agent to each other, preferably by way of a spacer moiety, and conjugating the resulting ligand/agent conjugate to the water soluble polymer. In particular embodiments, a biodegradable spacer moiety is used to form a linkage between the ligand and the chemical agent.

Peptide portions of the compositions of the present invention can be produced in a genetically engineered organism, such as *E. coli*, as a "fusion protein." That is, a hybrid gene containing a sequence of nucleotides encoding a ligand, spacer, or peptide chemical agent can be constructed by recombinant DNA technology. This hybrid gene can be inserted into an organism such that the "fusion protein" encoded by the hybrid gene is expressed. The fusion protein can then be purified by standard methods, including affinity chromatography. Peptides containing a ligand, spacer, or peptide chemical agent can also be constructed by chemical synthesis. Short peptide ligands are generally preferred, both because short peptides can be manipulated more readily and because the presence of additional amino acids residues, and particularly of substantial numbers of additional amino acids residues, may interfere with the function of the peptide ligand in stimulating internalization of the chemical agent by endocytosis.

Compositions according to the present invention preferably also further include a protease digestion site, such that once the composition is within the cell, such as in a lysosome, the chemical agent can be separated from the remainder of the composition by proteolysis of the digestion site. Such a protease susceptible

biodegradable peptide portion can be added regardless of whether the peptide portions of the composition are synthesized chemically or as expression peptides in a genetically engineered organism. In the latter case, nucleotides encoding the protease susceptible spacer can be inserted into the hybrid gene encoding the ligand and or a peptide chemical agent by techniques well known in the art. In one illustrative embodiment, the protease-susceptible peptide portion is designed to be cleaved by proteolysis in the lysosome of the target cell. The composition that is internalized by endocytosis is packaged in an endocytic vesicle, which is transported to a lysosome. Once in the lysosome, the protease-susceptible portion is cleaved, and the chemical agent is then available to be transported to the cytoplasm.

Another aspect of the present invention features a method for specifically effecting a desired activity in target receptor or IL-2-receptor-bearing cells, e.g. a cancer cell or activated T lymphocytes, contained in a heterogeneous population of cells, by the step of contacting the population of cells with a composition, prepared according to the present invention, that directs such activity into the cells. The compositions of the invention are selectively bound to cancer cells bearing a target receptor or IL-2-receptor-bearing T cells in the mixed population, whereupon endocytosis of the composition into such cells is stimulated, and the chemical agent effects its activity within such cells.

This application employs, except where otherwise indicated, standard techniques for manipulation of peptides and for manipulation of nucleic acids for expression of peptides. Techniques for conjugation of oligopeptides and oligonucleotides are known in the art, and are described for example in T. Zhu et al., 3 Antisense Res. Dev. 265 (1993); T. Zhu et al., 89 Proc. Nat'l Acad. Sci. USA 7934 (1992); P. Rigaudy et al., 49 Cancer Res. 1836 (1989), which are hereby incorporated by reference.

As is noted above, the invention features peptides, employed as ligands, spacers, and/or chemical agents. The peptides according to the invention can be made by any of a variety of techniques, including organic synthesis and recombinant DNA methods. Techniques for chemical synthesis of peptides are described, for example, in B. Merrifield et al., 21 Biochemistry 5020 (1982); Houghten, 82 Proc.

Nat'l Acad. Sci. USA 5131 (1985); M. Bodanszky & A. Bodanszky, The Practice of Peptide Synthesis (Springer-Verlag 2d ed., 1994), incorporated herein by reference. Techniques for chemical conjugation of peptides with other molecules are known in the art.

A fusion protein according to the invention can be made by expression in a suitable host cell of a nucleic acid containing an oligonucleotide encoding a ligand and/or spacer and/or chemical agent. Such techniques for producing recombinant fusion proteins are well-known in the art, and are described generally in, e.g., J. Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., 1989), the pertinent parts of which are hereby incorporated herein by reference. Reagents useful in applying such techniques, such as restriction endonucleases and the like, are widely known in the art and commercially available from any of several vendors.

Construction of compositions according to the present invention will now be described, with particular reference to examples in which a peptide ligand coupled and a cytotoxic chemical agent, adriamycin or doxorubicin, are coupled to a branched PEG or a pendent PEG.

Example 1

In this example, 7.8 g of branched, 8-arm PEG-COOH (20kDa; Shearwater Polymers, Inc., Huntsville, AL) and 0.25 g of p-nitrophenol were dissolved in 500 ml of tetrahydrofuran(THF) (Aldrich, making solution 3.6 mM with respect to the p-nitrophenol. The solution was cooled in an ice bath, and 0.9341 g of dicyclohexylcarbodiimide (DCC, Sigma) in THF was added to the reaction mixture in 4 aliquots. The reaction solution was stirred for 45 minutes in the ice bath. The temperature of the reaction solution was then raised to room temperature, and the reaction was then continued for another 101 hours. The reaction solution was then filtered through filter paper, and the filtrate was concentrated by evaporating the solvent with a rotary evaporator using a water pump. The clear concentrated solution (30 ml) was added to ether (750 ml). The precipitate was filtered, washed in ether, and dried in air. An aliquot of the product was dissolved in 0.1 N NaOH, and the concentration of the liberated p-nitrophenol was estimated by

spectrophotometry at 400 nm using a molar extinction coefficient of $\epsilon = 1.8 \times 10^4$ l/mol-cm. The product (PEG-Onp), was determined to have an ONp content of 201.3 μmol/g.

PEG-ONp(0.168 g, ONp content 201.3 μmol), prepared as described above, was dissolved in 2 ml anhydrous dimethylformamide (DMF), and 42.4 mg peptide TT23 (SEQ ID NO:22) was added to the solution. About 150 μl of triethylamine diluted 1:2 with DMF was added to the reaction mixture three times at 15 minute intervals, and then the solution was stirred for 17 hours at room temperature. The reaction solution was added to cold ether (300 ml), and the conjugate precipitates were filtered, washed with 200 ml ether, and dried. Amino acid analysis of the conjugate, PEG-T23-OH, showed 1 mole of peptide TT23 incorporated per mole of PEG.

Adriamycin (7.6MG, Sigma) and PEG-TT23-OH (85 mg) were dissolved in 2 ml DMF and DCC solid (14 mg) was added to the solution. The reaction was carried out for 17 hours, precipitated with 200 ml ether, filtered, and washed with ether. The precipitate was dried under vacuum and then dissolved in PBS buffer. The solution was dialyzed for 25 hours with 3 changes of PBS buffer. Adriamycin content of the product, PEG-TT23(SEQ ID NO:22)-ADR, was determined by spectrophotometry at 490 nm.

Example 2

A control composition having the formula PEG-Gly-Phe-Leu-Gly-ADR (hereinafter, "PEG-GFLG-ADR;" SEQ ID NO:21) was prepared according to the procedure of Example 1.

Example 3

A composition having the formula PEG-Gly-Leu-Glu-Arg-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (hereinafter, "PEG-TT7-ADR;" SEQ ID NO:14) was prepared according to the procedure of Example 1.

Example 4

A composition having the formula PEG-Gly-Leu-Glu^{tBl}-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:15), was prepared according to the procedure of Example 1.

Example 5

A composition having the formula PEG-Gly-Leu-Gln-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:16) was prepared according to the procedure of Example 1.

Example 6

A composition having the formula PEG-Gly-Leu-Asp-His-Ile-Phe-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:17) is prepared according to the procedure of Example 1.

Example 7

A composition having the formula PEG-Gly-Leu-Asn-His-Ile-Phe-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:18) is prepared according to the procedure of Example 1.

Example 8

A composition having the formula PEG-Thr-Gly-Leu-Gln-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (hereinafter, "PEG-TT15-ADR"; SEQ ID NO:19) was prepared according to the procedure of Example 1.

Example 9

A composition having the formula PEG-Ser-Leu-Gln-His-Ile-Leu-Leu-Gly-Phe-Leu-Gly-Adriamycin (SEQ ID NO:20) is prepared according to the procedure of Example 1.

Example 10

A composition having the formula PEG-Gly-Leu-Gln-His-Leu-Phe-Leu-Gly-Adriamycin (hereinafter, "PEG-TT13-ADR"; SEQ ID NO:13) was prepared according to the procedure of Example 1.

Example 11

A composition having the formula PEG-Thr-Gly-Leu-Asp-Arg-Ile-Leu-Leu-Adriamycin (hereinafter, "PEG-TT27-ADR";SEQ ID NO:24) is prepared according to the procedure of Example 1.

Example 12

A composition having the formula PEG-Thr-Gly-Leu-Asp-Arg-Leu-Leu-Leu-Adriamycin (SEQ ID NO:25) is prepared according to the procedure of Example 1.

Example 13

A composition having the formula PEG-Thr-Gly-Leu-Asn-Arg-Leu-Leu-Leu-Adriamycin (SEQ ID NO:26) is prepared according to the procedure of Example 1.

Example 14

A composition having the formula PEG-Thr-Gly-Leu-Asn-Arg-Ile-Leu-Leu-Adriamycin (SEQ ID NO:27) is prepared according to the procedure of Example 1.

Example 15

A composition having the formula PEG-Thr-Gly-Leu-Asp-Arg-Ile-Phe-Leu-Gly-Adriamycin (SEQ ID NO:28) is prepared according to the procedure of Example 1.

Example 16

A composition having the formula PEG-Thr-Gly-Leu-Asp-Arg-Leu-Phe-Leu-Gly-Adriamycin (SEQ ID NO:29) is prepared according to the procedure of Example 1.

Example 17

A composition having the formula PEG-Thr-Gly-Leu-Asn-Arg-Ile-Phe-Leu-Gly-Adriamycin (SEQ ID NO:30) is prepared according to the procedure of Example 1.

Example 18

A composition having the formula PEG-Thr-Gly-Leu-Asn-Arg-Leu-Phe-Leu-Gly-Adriamycin (SEQ ID NO:31) is prepared according to the procedure of Example 1.

Example 19

The *in vitro* effects of PEG-TT23(SEQ ID NO:22)-ADR prepared according to the procedure of Example 1, PEG-GFLG-ADR prepared according to the procedure of Example 2, and unconjugated adriamycin were tested on mouse CTLL-2 cells (ATCC No. TIB 214) as follows. CTLL-2 cells express the IL-2 high affinity receptor. Triplicate samples of 1 x 10^5 cells each were mixed with different concentrations of the purified compositions in 0.1 ml of culture medium (RPMI 1640, 10% fetal calf serum) in the wells of a 96-well microtiter plate (Falcon Microtest 111), and incubated for 48 hours at 37°C in a humidified, 5% CO_2 atmosphere. Thereafter, cell viability was assessed by a colorimetric method using the tetrazolium compound MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) and an electron coupling reagent, PMS (phenazine methosulfate). A.J. Cory et al., 3 Cancer Commun. 207 (1991); T.L. Riss & R.A. Moravec, 3 Mol. Biol. Cell. 184a (Supp.; 1992); T.M. Buttke et al., 157 J. Immunol. Methods 233 (1993), hereby incorporated by reference. MTS is bioreduced by living cells into a soluble formazan product. The absorbency of the formazan at 490 nm can be measured directly from 96 well

assay plates without additional processing. The quantity of formazan product as measured by the absorbency at 490 nm is directly proportional to the number of living cells in culture. Reagents for the MTS assay were obtained from Promega Corp. (Madison, Wisconsin). According to this method, 20 µl of MTS/PMS solution (Promega No. G-5421) was added to each well of the assay plate. The plate was then further incubated at 37°C in a humidified, 5% CO_2 atmosphere for 4 hours. The absorbency of each well was then measured at 490 nm with an EL311 Microplate Autoreader (Bio-Tek Instruments). The mean absorbency for treatment was then calculated, and the percent cytotoxicity was determined using the formula:

$$\% \ cytotoxity = (1 - \frac{A_S}{A_C}) \times 100$$

wherein A_S represents the mean absorbency for each treatment and A_C represents mean absorbency of the control treatment, *i.e.* cells not exposed to a conjugate.

FIG. 1 shows that PEG-TT23-ADR (◊) kills such CTLL-2 T cells at concentrations much lower than that required for PEG-GFLG-ADR (△) to effect similar levels of cytotoxicity. These results show that the presence of a ligand specific for binding to the IL-2 receptor and inducing receptor-mediated endocytosis results in much greater cytotoxicity than a PEG- and adriamycin-containing conjugate lacking such ligand. Thus, a conjugate bearing an IL-2-receptor specific ligand is internalized with much greater efficiency that similar conjugates lacking such a ligand. The unconjugated adriamycin control(o) rapidly diffuses into the cells and kills them. As expected, cytotoxicities from PEG-TT23-ADR require higher concentrations of adriamycin than unconjugated adriamycin due to the requirement that PEG-TT23-ADR be internalized by endocytosis.

Example 20

The *in vitro* effects of IL-2-receptor-targeted conjugates on IL-2 induced proliferation of murine splenocytes was examined.

A sterile splenocyte suspension was prepared as follows. Spleens were aseptically removed from male C57 BL/6 mice and placed in sterile tissue culture

medium (RPMI plus 10% fetal calf serum). The spleens were teased apart and gently aspirated using a Pasteur pipet. The resulting spleen cell suspension was then filtered through sterile gauze and centrifuged at 150 x *g* for 10 minutes at 20°C. The supernate was discarded, and erthrocytes in the cell pellet were selectively lysed by resuspending the pellet in lysis buffer (155 mM NH_4Cl, 13.41 mM $KHCO_3$, and 100 mM EDTA in dH_2O). After 30 seconds, an equal volume of tissue culture medium was added to the suspension to restore isotonicity. The cell suspension was again centrifuged at 150 x *g* for 10 minutes at 20°C, and the resulting cell pellet was washed once using tissue culture medium. The washed cell pellet was finally resuspended in tissue culture medium, and cell density was adjusted to 5 x 10^6 cells/ml.

To the wells of a 96-well assay plate (Falcon MICROTEST III), 50 μl volumes of the splenocyte suspension were added. Competitor (conjugate) solutions were prepared and diluted in tissue culture medium and added to the wells of the assay plate in 25 μl volumes. To control wells, 25 μl of tissue culture medium was added. The assay plate was then incubated for 60 minutes at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. To the wells of the assay plate, 25 μl of either tissue culture medium or a solution containing recombinant human IL-2 (Pharmingen) at a concentration of 20 ng/ml (in tissue culture medium) was added. The assay plate was then incubated for 48 hours at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air.

Final results were quantitated as follows. Viable cell counts were performed for each separate well of the assay plate using trypan blue exclusion method. Trypan blue is taken up and imparts a blue color to dead cells. Briefly, an aliquot of cells was twice diluted in 0.4% trypan blue stain (Sigma Chemical Co., St.Louis, Missouri) and incubated for 5 minutes before counting with a hemacytometer and an inverted microscope. The percentage of viable cells was calculated as the number of unstained cells per unit volume divided by the total number of stained and unstained cells x 100. Cell counts for each duplicate set of wells were averaged, and cell proliferation was calculated as percent change from control cells using the following formula:

$$\% \text{ Change} = \left[\frac{\text{mean cell count, test}}{\text{mean cell count, control}} - 1\right] \times 100$$

Percent inhibition of IL-2 induced proliferation was calculated as follows.

$$\% \text{ Inhibition} = \frac{[(\% \text{ Change}_{+IL\text{-}2}) - (\% \text{ Change}_{IL\text{-}2 + \text{competitor}})]}{\% \text{ Change}_{+IL\text{-}2}} \times 100$$

The materials tested in this example was PEG-TT23, prepared according to the procedure of Example 1 except that no adriamycin was conjugated thereto, and unconjugated peptide TT23.

The results of conjugate inhibition of IL-2-induced proliferation of murine splenocytes with PEG-TT23 and peptide TT23 are shown in Figure 2. These results show that PEG-TT23 very effectively inhibits proliferation, whereas about 100-fold more unconjugated peptide TT23 is needed to achieve similar levels of inhibition. These results show that conjugate PEG-TT23 specifically binds to the IL-2 receptor.

The compositions according to the present invention can be employed for targeted delivery of a chemical agent to IL-2-receptor-bearing cells, e.g. activated T cells, generally by contacting the cells with the composition under conditions in which binding of the ligand to a receptor stimulates endocytosis of the composition into the cells. The chemical agent then acts on or within the targeted cell into which the composition is internalized, and the desired effect of the active agent can be defined to those cells having the receptor.

For example, a composition according to the invention can be employed as an effective antitumor agent *in vivo* for killing cancer cells and/or activated T cells. The composition can also be used for treating cancer and/or T-cell-associated diseases and tissue graft rejection. Such diseases include cancer, arthritis, cutaneous T-cell lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, skin cancers, psoriasis, graft rejection disease, multiple sclerosis, Type II diabetes mellitus, and disease resulting from HIV infection. The composition can be administered locally or systemically. Preferably, the composition is administered to the subject by

systemic administration, typically by subcutaneous, intramuscular, or intravenous injection, or intraperitoneal administration, which are methods well known in the art. Injectable preparations for such use can be made in conventional forms, either as a liquid solution or suspension or in a solid form suitable for preparation as a solution or suspension in a liquid prior to injection, or as an emulsion. Suitable excipients include, for example, water, saline, dextrose, glycerol, ethanol, and the like; and if desired, minor amounts of auxiliary substances such as wetting or emulsifying agents, buffers, and the like may be added. Effective amounts of such compositions can be determined by those skilled in the art without undue experimentation according to the guidelines provided herein.

The composition can be contacted with the cells *in vitro* or *in vivo*. The T cells constitute a subpopulation of a mixed population of cell types; the ligand according to the invention can provide for endocytosis of the conjugate into T cells and possibly into a small proportion of other cells having a closely related receptor.

The chemical agent can have any of a variety of desired effects in the targeted cells. As mentioned above, in some particularly useful embodiments the chemical agent is effective on a cell only when, or principally when, the agent is internalized into the cell.

Example 21

Treatment of Collagen-Induced Arthritis (CIA)

Collagen-induced arthritis (CIA) is a reliable model for pre-clinical testing of new immunomodulatory drugs for rheumatic diseases. CIA is induced in genetically susceptible mice by immunization with heterologous type II collagen (CII). The severity of CIA reflects the level of autoreactive T cells sensitized to CII, the production of CII-reactive autoantibody and the release in joint tissues of a variety of pro-inflammatory cytokines and chemokines. Treatment protocols that interfere with the T cell activation step inhibit CIA.

In this example, activated T cells were targeted with PEG-TT23-ADR, which has been shown to specifically bind to the IL-2 receptor (Example 20). The IL-2 receptor is only expressed by activated T cells. Thus, resting T cells are

spared, and the intracellular toxic effects of the ADR-conjugate are directed to CIL-reactive T cells.

B10.RIII mice were injected with CII (200 μg) emulsified with complete Freund's adjuvant (CFA;200 μg H37Ra). At day 21, mice were boosted with 100 μg of CII, randomized for CIA severity (13/group), and an alternate day treatment protocol was begun. This secondary boost with CII is known to cause a rapid precipitation of arthritis onset. After such a secondary boost, B10.RIII mice show a very rapid onset of arthritis and very severe diseases with a >90% incidence. Test groups received by i.p. injection: (A) PEG-TT23-ADR (2 mg/kg); (B) PEG-GFLG-ADR (2 mg/kg); (C) PBS (vehicle control). Mice were observed for 84 days for arthritis severity and weight changes.

Group B showed a significant weight loss of about 10% ± 8% as compared to Group C (about 5% ± 5 %) $P \leq 0.04$, that was not evident in Group A mice (about 3% ± 6%) $P \leq 0.26$. Distribution curves of the cumulative daily arthritis scores revealed significant differences ($P \leq 0.02$) among the 3 groups. Importantly, Group A mice showed a significantly greater number of mice with only minimum arthritis as compared to the normally severe disease found in the majority of PBS control mice ($P \leq 0.02$).

Several potentially beneficial drugs for rheumatoid arthritis are contra-indicated by toxicity at required dosages. These data suggest that delivering a specific therapeutic agent only to the particular subset of lymphocytes directly involved in disease pathology may allow effective therapy to be accomplished at lower, more tolerable dosages.

Example 22

A method of treating T cell lymphoma in a human comprises (a) providing a composition according to the present invention, such as PEG-TT23-ADR (SEQ ID NO:22), wherein the chemical agent is a cytotoxin, and (b) systemically administering an effective amount of the composition to an individual. An effective amount of the composition is systemically administered to the individual such that the composition enters the bloodstream and contacts T

cells. The composition binds to an IL-2 receptor on the T cells and stimulates internalization of the composition by endocytosis. The biodegradable spacer is digested by intracellular proteases, releasing the adriamycin. The adriamycin then kills the cell by intercalating with DNA in the cell. This procedure reduces the number of malignant T cells in the body of the individual, thereby having a positive effect in treatment of the disease.

Example 23

Alterations in the immune status of patients with various cancers results in release of soluble IL-2 receptors (sIL-2R) in circulation. The sIL-2R levels in T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, and peripheral T-cell lymphoma are an indication of the degree of T-cell or immune activation due to concomitant immunologic processes in these disorders. S. Raziuddin et al., 73 Cancer 2426-2431 (1994). Further, sIL-2R levels remain elevated in Hodgkin's disease and non-Hodgkin's lymphoma patients, even at the stage of minimal residual disease after intensive chemotherapy or radiotherapy. M. Kandefer-Szenrzen et al., 45 Arch. Immunol. Ther. Exp. (Warsz) 443-448 (1997). Therefore, detection of elevated levels of sIL-2R in circulation can be used as a diagnostic assay for such cancers.

In this example, compositions according to the present invention are used for detecting sIL-2R in circulation according to methods well known in the art. Peptides that bind to sIL-2R are used in modified ELISA or RIA assays for detecting such sIL-2R in circulation. The peptides are labeled with an enzyme or with a radiolabel. The labeled peptide is then mixed with a sample, such as a serum sample, and the mixture is incubated under conditions suitable for binding of the peptide to the sIL-2R to form a complex. This complex is then detected by colorimetric, fluorometric, radiometric, or similar assay. Elevated levels of circulating sIL-2R indicates the presence of cancer.

Example 24

Preparation of pendant 20 KD PEG-8PA-ONp

Purification of Starting Material: Innopeg 20M-8PA (15 g, Innophase Corporation) was dissolved in 100 ml of 5% water in methanol and the solution introduced into a Spectra/Por MWCO 12-14,000 dialysis bag and the solution dialyzed against 2 L of 5% aqueous methanol for 24 hours. The dialysate was replaced with fresh 5% aqueous methanol and dialysis continued 24 hours. The process was repeated one additional time and the material in the bag concentrated to a thick syrup. The majority of the solvent was removed at 80°C on the rotary evaporator using a vacuum pump and the glass recrystallized from 180 ml of acetone to yield 8.37 g of purified 20KD pPEG-8PA. The combined dialysates after concentration *in vacua* amounted to 5.59 g or 37.2% of the total. Before dialysis the polymer was determined to contain an average of 8.3 carboxyl groups per mole polymer by titration with 0.01N NaOH solution, while after dialysis the number was reduced to 6.5 carboxyl groups per mole. Even though the purified starting material contained 6.5 carboxyls per mole polymer, it is still referred to as 20KD pPEG-8PA.

Reaction and Work-up (non-extractive): A 250 ml round bottom flask, equipped with magnetic stirring bar, distillation head and condenser was charged with 15.0 g (MW = 19,000 by GPC, 789.5 micromoles) of 20KD polyethylene glycol propionic acid (20KD-pPEG-8PA, dialyzed and recrystallized, 6.5 pendant carboxyl groups/mole polymer). Anhydrous toluene (150 ml) was added and heat was applied via a heating mantle to the rapidly stirred suspension. As the temperature increased, the starting material dissolved. Approximately 60 ml of toluene was distilled from the solution over a 30 minute period to remove any water. The remainder of the toluene was removed *in vacua* on a rotary evaporator using a water aspirator, and finally, traces of toluene were removed at 75°C on the rotary evaporator using a vacuum pump. The glassy syrup was dissolved in 150 ml of anhydrous dichloromethane and 1.62 g (11.66 mmole) of p-Nitrophenol (recrystallized twice from toluene) added followed by 150 mg (1.23 mmole) of 4-(dimethylamino) pyridine (DMAP). Finally, 2.24 g (11.68 mmole) of finely ground 1-[3-(dimethylamino)propel]-3-ethyl carbodiimide (EDC) was added in portions over a period of 30 minutes to the stirred solution

under a nitrogen atmosphere. The reaction was stirred at room temperature for 4 hours. After the reaction period, glacial acetic acid (361 mg, 6.01 mmole, 345 microliters) was added to react with the excess p-nitrophenol and EDC, and stirring was continued 30 minutes. Finally, the DMAP catalyst was deactivated by the addition of 234 mg (1.23 mmole) of p-toluenesulfonic acid monohydrate and the solution concentrated *in vacua* to a thick syrup at 40°C and finally cooled to room temperature.

Purification: Methanol (MeOH, 20 ml, more can be added if necessary) was added and the material stirred until complete dissolution of the glass had occurred. Isopropanol (IPA, 50 ml) was added and the solution cooled slightly with cold tap water. After a few minutes, a precipitate formed on the bottom of the flask. The solution containing the precipitate was stirred at room temperature while adding 150 ml IPA over a period of 5 minutes. The material was digested by stirring at room temperature a minimum of 30 minutes, cooling to 0°C in an ice bath and finally to -15°C in an ice/salt bath. The solid was filtered, washed with 80 ml of IPA at -15°C in several portions and air dried on the Buchner funnel to a damp solid (until no more IPA passed through the funnel). The solid was quantitatively removed from the Buchner funnel and re-purified as above using the same quantities of MeOH and IPA. After the second recrystallization/precipitation, the filter cake was washed with 25 ml of ether in three portions. The air dried material was powdered by passing through a 200 mesh sieve and the product vacuum dried overnight at room temperature to yield 15.09 g (97.1%) of 20KD pPEG-8PA-ONp which was determined to contain 5.5 moles of ONp/mole polymer. The product was analyzed by TLC using 10/1/0.25 $CHCl_3$/MeOH/AcOH (Merck. aluminum backed F-254 silica gel plates) and the plate first visualized under UV light. Only material at the origin could be seen as a dark spot. The plate was then dipped into concentrated ammonium hydroxide solution to visualize p-nitrophenol containing molecules. Only material at the origin developed the characteristic yellow color of p-nitrophenoxide anion. No free p-nitrophenol or p-nitrophenyl acetate could be detected in the product. Finally, the plate was strongly heated with a heat gun to drive off volatile

materials, dipped into a 1.5% methanolic solution of silver nitrate and again strongly heated with the heat gun. A dark brown spot developed at the origin. No other contaminants were present. The product should be stored at -20°C and converted to the next intermediate within a 5-day period in order to minimize further chain elongation and cross-linking.

Example 25

Preparation of Pendant 20KD pPEG-8PA-TT45

Reaction and Work-up: 20KD pPEG-8PA-PNp (MW = 19,650, 3.0 g, 152.7 micromoles and TT45(SEQ ID NO:31) [1030 mg, 686.2 micromoles, 4.5 equivalents (as the bis-trifluoroacetate salt, MW = 1501)] were dissolved in 30 ml of DMF and 127 mg (1041 micromoles, 6.8 equivalents) of DMAP added. When complete dissolution of all solids was obtained, 134 mg (1041 micromoles, 181 microliter, 6.8 equivalents) of DIEA were added and the solution stirred at room temperature overnight. The reaction mixture was treated with 200 microliters of concentrated ammonium hydroxide for 30 minutes, 9.0 ml of glacial acetic acid added and the solution concentrated *in vacuo* at 50°C using a vacuum pump on a rotary evaporator.

Purification by LH-20 Chromatography: The residue from above was dissolved in 50 ml of MeOH and introduced onto the top of a 5 x 65 cm LH-20 column packed in MeOH. The flask containing the original MeOH solution was rinsed three times with a ~ 10 ml of MeOH and the washings introduced onto the column. The solution at the top of the column was stirred with a spatula without disturbing the bed until homogeneous and then allowed to flow onto the column such that the liquid level was just above the bed and the flow stopped. The sample was allowed to equilibrate with the column for 15 to 30 minutes (preferably 20 minutes) before the flow was again started (flow rate = 10 ml/min.). Fresh solvent was added to the column, fractions collected, and analyzed by TLC (acetonitrile/n-butanol/toluene/acetic acid/water 1/1/1/1/1. ABTAW). The product eluted between 460 and 775 ml. The absence of free TT30 in samples containing product was established by visualization with both

UV light and ninhydrin plus heat, while UV light alone confirmed the absence of DMAP and p-nitrophenol (plate can be dipped in concentrated ammonium hydroxide to confirm). Finally, visualization of the plate with 1.5% methanolic silver nitrate plus heat showed the presence of material at the origin in fractions containing only the product. Fractions were combined, concentrated *in vacua* and the residue dissolved in a minimum amount of MeOH. Ether (200 ml) was added in one portion to the rapidly stirred methanol solution and the suspension stirred at room temperature 1 hour. The suspension was, filtered, the filter cake washed with ether, air dried and vacuum dried overnight at room temperature to afford 3.53 g of 20KD pPEG-8PA-TT30. A nitrogen determination on this product showed it to contain 3.91%N. Typically between 4.2 and 4.4 moles of TT45/mole polymer is incorporated using the above procedures.

Example 26

Preparation of Pendant 20KD pPEG-8PA-TT45(SEQ ID NO:31)-DXR

Reaction and Work-up: To a solution of 2.0g (81.14 micromoles) of 20KD pPEG-8PA-TT45 (MW = 24,650, 4.25 mole TT45/mole polymer) and 188 mg of DXR HCl(324.5 micromoles, 4 equivalents) in 20 ml of anhydrous DMF was added 41.8 mg (324.6 micromoles, 56 microliters, 4 equivalents) of DIEA followed by 90.2 mg (649 micromoles, 8 equivalents) of p-nitrophenol and 19.8 mg (162.3 micromoles, 2 equivalents) of DMAP. EDC (124.4 mg, 649 micromoles, 8 equivalents) was added and finally another 41.8 mg (324.6 micromoles, 57 microliters) of DIEA. The reaction was stirred at room temperature for 18 hours under a nitrogen atmosphere. The reaction was treated with 50 microliters of glacial acetic acid for 60 minutes before concentrating *in vacua* at 45 to 50°C on a rotary evaporator equipped with a vacuum pump.

Purification by LH-20 Chromatography: The residue was dissolved in 50 ml of methanol and introduced into the center of a 5.5 x 65 cm column of LH-20 packed in MeOH. A piece of shark-skin filter paper was placed on top of the column to prevent the bed from being distributed. The flask containing the sample was rinsed three times with ~10 ml of MeOH, the washings introduced

onto the column, and the solution stirred with a spatula until homogeneous. The sample was allowed to flow onto the column such that the liquid level was just above the bed and the flow stopped. The sample was allowed to equilibrate on the column for 20 minutes and the flow re-initiated by introducing fresh MeOH according to standard procedures (flow-rate = 9.0 ml/min). As the separation proceeded, the drug conjugate moved quickly through the column eluting it between 350 and 550 ml. Some trailing into lower molecular weight components was observed. The product appeared well separated from unreacted doxorubicin and doxorubicin acetamide formed during the quench; however, TLC analysis of the fractions containing the product showed the presence of small amounts of free doxorubicin. The fractions were combined and concentrated and the residue replaced in the freezer while cleaning the column.

As mentioned earlier, the column is cleaned using MeOH containing 0.6% concentrated hydrochloric acid. (11 of MeOH containing 6.0 ml of concentrated hydrochloric acid). For the intermediate size column (5.5 x 65 cm), generally, 1 L of the acid solution followed by enough MeOH to bring the pH of a solution of 2 ml of the effluent in 10 ml of water to the same or higher ph of 2 ml of reagent grade MeOH in 10 ml of water. In addition, it is a good idea to collect ~300 to 500 ml of effluent after achieving the proper pH, concentrating it down, reconstituting in 10 ml of MeOH and obtaining the absorbency of the solution at 488 nm (ϵ = 10,000) to be sure that significant amounts of doxorubicin containing compounds are absent. In the case of the larger 6.5 x 65 cm column 1.51 of the acid should be used for cleaning. It is important that once the acid is introduced onto the column, the cleaning procedure should be continued until the column is clean and the pH adjusted to 6.5. Acid should not be allowed to sit on the column overnight or for longer periods of time, for example. Of course, the methanol can also be recycled.

The sample was re-chromatographed exactly as above. Analysis of the combined fractions by TLC (ABTAW) shoed the absence of free doxorubicin or doxorubicin acetamide. The combined sample was concentrated *in vacuo* and the dark red glass dissolved in 35 ml of water. After filtering the solution through a

0.45 micron microporous filter and rinsing the apparatus with a small amount of water, the solution was frozen in a dry-ice/acetone bath and lyophilized overnight. The almost brick-red product weighed 1.20 g*. The product was found to contain 4.19 wt.% doxorubicin or .96 moles of DXR per mole of polymer by measuring the UV absorption at 488 nm ϵ = 10,000) in PBS buffer at pH 7.2. Of the 4.19 wt.% total doxorubicin 3.89% of this or 0.16% represents "free doxorubicin".

Example 27

The *in vitro* cytotoxicity dose response curves of 20KD pPEG-8PA-TT45-DXR prepared according to the procedure of Example 26, and unconjugated Doxorubicin(DXR) were measured on three human cell lines: Human B-cell lymphoma cell line(Raji and Daudi), Human T-cell lymphoma cell line(Hut78), according to a procedure described in Example 19.

The results are shown in FIG.3 and FIG 4. As expected, cytotoxicities from 20KD pPEG-8PA-TT30-DXR require higher concentrations of doxorubicin(FIG 3) than unconjugated Doxorubicin(FIG 4) due to the requirement that pPEG-8PA-TT30-DXR be internalized by endocytosis, while the unconjugated Doxorubicin control rapidly diffuses into the cells and kills them. These results show that the presence of a ligand specific for binding to the IL-2 receptor and inducing receptor-mediated endocytosis in the three human cell lines tested, thus reducing side effects caused by administration of free doxorubicin.

The above examples are presented to enable those skilled in the art to understand more clearly and practice the present invention. It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that which follows is intended to illustrate and not limit the scope of the invention. Other aspects of the invention will be apparent to those skilled in the art to which the invention pertains.

CLAIMS

We claim:

1. A composition for intracellular delivery of a chemical agent into a target-receptor bearing cell, the composition comprising (a) a water-soluble, biocompatible polymer, (b) a chemical agent covalently, releasably coupled to the polymer, and (c) at least one copy of a ligand comprising a target-receptor-binding peptide covalently coupled to the polymer, wherein the ligand stimulates internalization thereof by receptor-mediated endocytosis, the ligand being biodegradable and the active agent being releasable into the targeted cell following endocytosis.

2. The composition of claim 1, wherein the target receptor is an IL-2-receptor.

3. The composition of claim 1, wherein the target receptor is a cancer cell bearing receptor, other than an Il-2-receptor, and capable of binding to a composition comprising a ligand selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof.

4. The composition of claim 2 or 3, wherein the ligand is a peptide containing a biodegradable portion.

5. The composition of claim 2 or 3 wherein the biocompatible polymer is a polyalkylene oxide.

6. The composition of claim 5 wherein said polyalkylene oxide is a member selected from the group consisting of alpha-substituted polyalkylene oxide derivatives, polyethylene glycol homopolymers and derivatives thereof, polypropylene glycol homopolymers and derivatives thereof, alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), branched polyethylene glycols, star polyethylene glycols, pendent

polyethylene glycols, block copolymers of poly(alkylene oxides) and the activated derivatives thereof.

7. The composition of claim 6 wherein said polyalkylene oxide has a molecular weight of about 200 to about 50,000.

8. The composition of claim 7 wherein said polyalkylene oxide has a molecular weight of about 2,000 to about 20,000.

9. The composition of claim 8 wherein said polyalkylene oxide has a molecular weight of about 20,000.

10. The composition of claim 7 wherein said polyalkylene oxide is an activated polyethylene glycol.

11. The composition of claim 7 wherein said polyalkylene oxide is a pendent or star polyethylene glycol.

12. The composition of claim 7 wherein said polyalkylene oxide is a branched polyethylene glycol.

13. The composition of claim 7 wherein said polyalkylene oxide is polyethylene oxide.

14. The composition of claim 1 wherein said target receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47 and biologically functional equivalents thereof..

15. The composition of claim 14 wherein said target receptor-binding peptide is SEQ ID NO:22 or a biologically functional equivalents thereof.

16. The composition of claim 14 wherein said target receptor-binding peptide is SEQ ID NO:31 or a biologically functional equivalents thereof.

17. The composition of claim 14 wherein said target receptor-binding peptide is SEQ ID NO:46 or a biologically functional equivalents thereof.

18. The composition of claim 14 wherein said biodegradable portion comprises Gly-Phe-Leu-Gly (SEQ ID NO:21) or a biologically functional equivalents thereof.

19. The composition of claim 1 wherein said chemical agent is selected from the group consisting of cytotoxins, immunosuppressants, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

20. The composition of claim 19 wherein said chemical agent is a cytotoxin selected from the group consisting of adriamycin, doxorubicin, taxol, ~~cis~~platin, methotrexate, cyclophosphamide and derivatives thereof.

21. The composition of claim 20 wherein said chemical agent is a member selected from the group consisting of adriamycin, doxorubicin and derivatives thereof.

22. The composition of claim 19 wherein said chemical agent is an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK506 and derivatives thereof.

23. The composition of claim 1 further comprising a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates.

24. The composition of claim 23 wherein said carrier is a water soluble polymer selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid), and polymers and copolymers of N-substituted methacrylamide.

25. The composition of claim 2 wherein said target-receptor-bearing cell is an activated T cell.

26. The composition of claim 3 wherein said target-receptor-bearing cell is a cancer cell.

27. A composition comprising a peptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47, amides and other chemical modifications that result in biologically functional equivalents thereof.

28. The composition of claim 27 wherein the peptides selected from the group consisting of SEQ ID NO:22, SEQ ID NO:31, and SEQ ID NO:46.

29. The composition of claim 28 wherein the peptides selected from the group consisting of SEQ ID NO:22, SEQ ID NO: 31.

30. A method of delivering a chemical agent *in vitro* into a target-receptor-bearing cell in a population of cells, comprising the steps of:

(a) providing a composition comprising (i) a water-soluble, biocompatible polymer, (ii) the chemical agent covalently, releasably coupled to the polymer, and (iii) at least one copy of a ligand comprising a target-receptor-binding peptide covalently coupled to the polymer, wherein the ligand stimulates internalization thereof by receptor-mediated endocytosis, the ligand being biodegradable and the active agent being releasable into the targeted cell following endocytosis; and

(b) contacting the population of cells with an effective amount of the composition under conditions wherein the ligand binds to a target receptor on the target-receptor-bearing cells and elicits endocytosis of the composition into the cells.

31. A method of delivering a chemical agent into a target-receptor-bearing cell in a warm-blooded animal, comprising the steps of:

(a) providing a composition comprising (i) a water-soluble, biocompatible polymer, (ii) a chemical agent covalently, releasably coupled to the polymer, and (iii) at least one copy of a ligand comprising an target-receptor-binding peptide covalently coupled to the polymer, wherein the ligand stimulates internalization thereof by receptor-mediated endocytosis and the ligand being biodegradable and the active agent being releasable into the targeted cell following endocytosis; and

(b) administering to said warm-blooded animal an effective amount of the composition under conditions wherein the ligand contacts and binds to a target receptor on the target-receptor-bearing cells and elicits endocytosis of the composition.

32. The method of claim 31, wherein the target receptor is an IL-2-receptor.

33. The method of claim 31, wherein the target receptor is a cancer cell bearing receptor, other than an Il-2-receptor, and capable of binding to a composition comprising a ligand selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof.

34. The method of claim 32 or 33, wherein the ligand is a peptide containing a biodegradable portion.

35. The method of claim 34, wherein the biocompatible polymer is a polyalkylene oxide.

36. The method of claim 35 wherein said polyalkylene oxide is a member selected from the group consisting of alpha-substituted polyalkylene oxide derivatives, polyethylene glycol homopolymers and derivatives thereof, polypropylene glycol homopolymers and derivatives thereof, alkyl-capped polyethylene oxides, bis-polyethylene oxides, copolymers of poly(alkylene oxides), branched polyethylebe glycols, star polyethylene glycols, pendent polyethylene glycols, block copolymers of poly(alkylene oxides) and the activated derivatives thereof.

37. The method of claim 36 wherein said polyalkylene oxide has a molecular weight of about 200 to about 50,000.

38. The method of claim 37 wherein said polyalkylene oxide has a molecular weight of about 2,000 to about 20,000.

39. The method of claim 38 wherein said polyalkylene oxide has a molecular weight of about 20,000.

40. The method of claim 37 wherein said polyalkylene oxide is an activated polyethylene glycol.

41. The method of claim 37 wherein said polyalkylene oxide is a pendent or star polyethylene glycol.

42. The composition of claim 37 wherein said polyalkylene oxide is a branched polyethylene glycol.

43. The method of claim 37 wherein said polyalkylene oxide is polyethylene oxide.

44. The method of claim 34 wherein said target-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 and biologically functional equivalents thereof.

45. The method of claim 44 wherein said target receptor-binding peptide is a member selected from the group consisting of group consisting of SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47 and biologically functional equivalents thereof.

46. The composition of claim 44 wherein said target receptor-binding peptide is SEQ ID NO:22 or a biologically functional equivalent thereof.

47. The composition of claim 44 wherein said target receptor-binding peptide is SEQ ID NO:31 or a biologically functional equivalent thereof.

48. The composition of claim 44 wherein said target receptor-binding peptide is SEQ ID NO:46 or a biologically functional equivalent thereof.

49. The composition of claim 44 wherein said biodegradable portion comprises Gly-Phe-Leu-Gly (SEQ ID NO:21) or a biologically functional equivalent thereof.

50. The method of claim 44 wherein said chemical agent is selected from the group consisting of cytotoxins immunosuppressants, transforming nucleic acids, gene regulators, labels, antigens, and drugs.

51. The method of claim 50 wherein said chemical agent is a cytotoxin selected from the group consisting of adriamycin, doxorubicin, taxol, cisplatin, methotrexate, cyclophosphamide and derivatives thereof.

52. The method of claim 51 wherein said chemical agent is adriamycin, doxorubicin and derivatives thereof.

53. The composition of claim 51 wherein said chemical agent is an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK506 and derivatives thereof.

54. The method of claim 31 further comprising a carrier selected from the group consisting of other water soluble polymers, liposomes, and particulates.

55. The method of claim 54 wherein said carrier is a water soluble polymer selected from the group consisting of dextran, inulin, poly(L-lysine) with modified epsilon amino groups, poly(L-glutamic acid), and polymers and copolymers of N-substituted methacrylamide.

56. The method of claim 32 wherein said target-receptor-bearing cell is an activated T cell.

57. The method of claim 33 wherein said target-receptor-bearing cell is a cancer cell.

58. The method of claim 31, wherein the administering step is conducted by a systemical means selected from the group consisting of subcutaneous, intramuscular, or intravenous injection, and intraperitoneal administration.

59. The method of claim 31, wherein the administering step

is conducted by a local administering means.

60. A method of detecting a disease associated with elevated levels of soluble target receptor in circulation comprising the steps of:

(a) providing a composition of a target-receptor-binding peptide; and

(b) mixing said composition with a body fluid to be tested under conditions suitable for binding of said composition to said soluble target receptor on the target-receptor in said body fluid to form a complex; and

(c) detecting said complex and determining whether said complex is present at elevated levels as compared to normal individuals.

61. The method of claim 60 wherein said disease is a member selected from the group consisting of T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma.

62. The method of claim 60 wherein said disease is a cancer.

63. The method of claim 60 wherein said body fluid is serum.

64. The method of claim 60 wherein said detecting comprises an enzymatic or radioactive sorbent assay.

65. The method of claim 60 wherein said target-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:22 through SEQ ID NO:47, amides thereof and biologically functional equivalents thereof.

66. The method of claim 65 wherein said target-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:46 and amides thereof.

67. The method of claim 66 wherein said target-receptor-binding peptide is a member selected from the group consisting of SEQ ID NO:22, SEQ ID NO:31, amides thereof and biologically functional equivalents thereof.

68. The method of claim 66 wherein said target-receptor-binding peptide is SEQ ID NO:31 or biologically functional equivalents thereof.

69. The method of claim 66 wherein said target-receptor-binding peptide is the amide of SEQ ID NO:31 or biologically functional equivalents thereof.

70. The method of claim 66 wherein said target-receptor-binding peptide is SEQ ID NO:22 or biologically functional equivalents thereof.

71. The method of claim 70 wherein said target-receptor-binding peptide is the amide of SEQ ID NO:22 or biologically functional equivalents thereof.

72. The method of claim 66 wherein said target-receptor-binding peptide is SEQ ID NO:46 or biologically functional equivalents thereof.

73. The method of claim 72 wherein said target-receptor-binding peptide is the amide of SEQ ID NO:46 or biologically functional equivalents thereof.

74. A composition comprising a conjugate of a pendent PEG and a peptide.

75. A composition comprising a conjugate of a star PEG and a peptide.

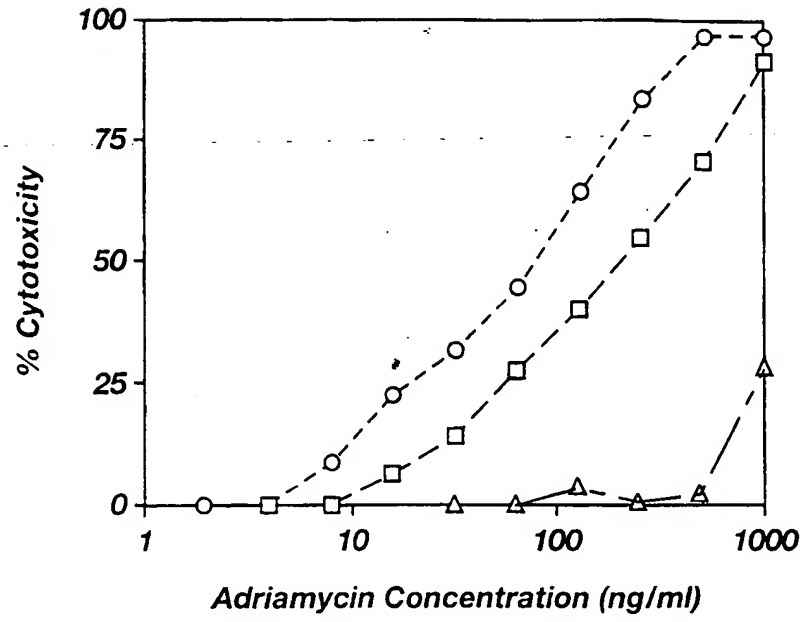
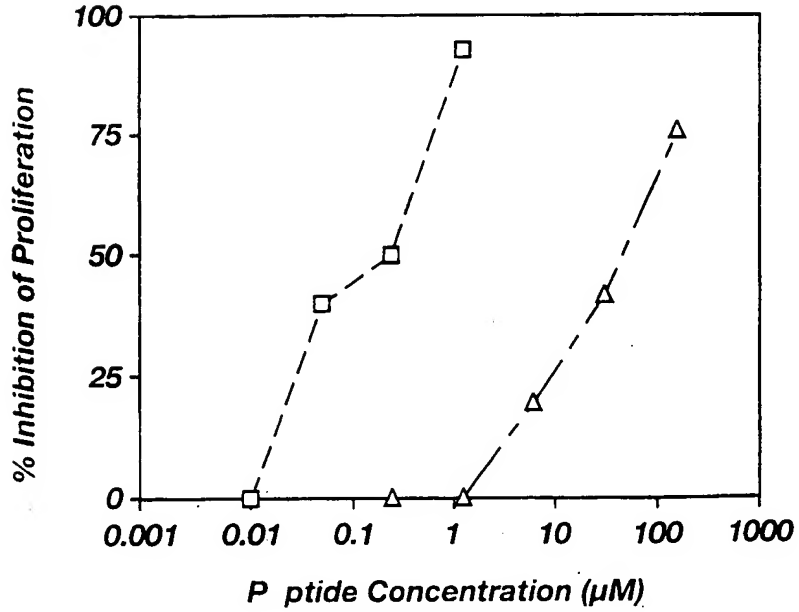

100
75
50
25
0
% Cytotoxicity
1
10
100
1000
Adriamycin Concentration (ng/ml)

Fig. 1


100
75
50
25
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0.01
0.1
1
10
100
1000
P ptide Concentration (μM)

Fig. 2

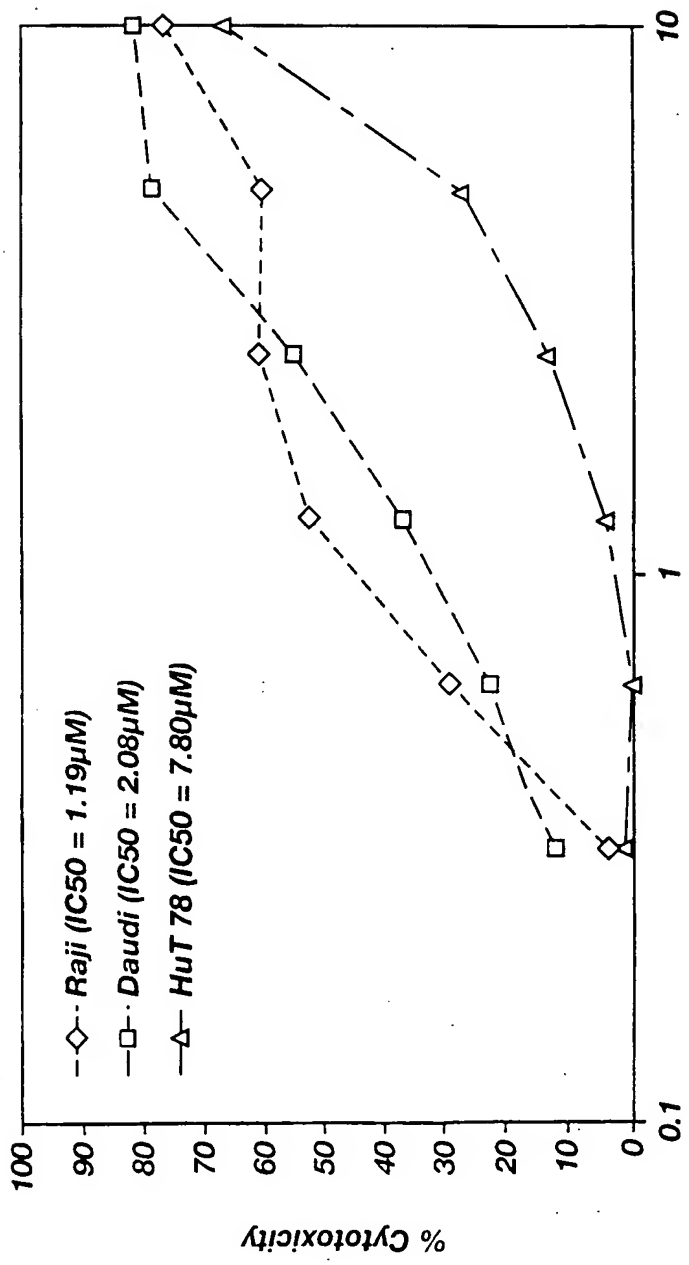
% Cytotoxicity
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
DXR Concentration (μM)
Raji (IC50 = 1.19μM)
Daudi (IC50 = 2.08μM)
HuT 78 (IC50 = 7.80μM)

Fig. 3

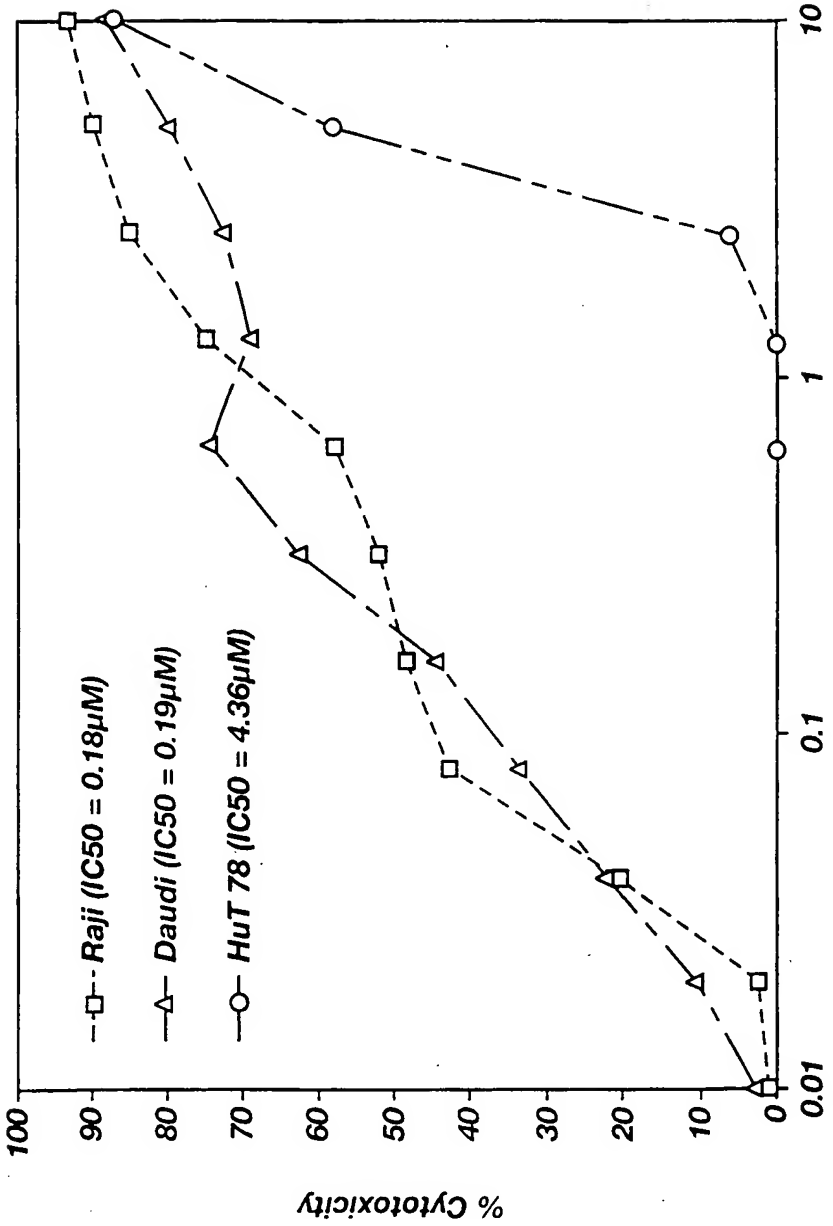
% Cytotoxicity
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
Doxorubicin Concentration (μM)
Raji (IC50 = 0.18μM)
Daudi (IC50 = 0.19μM)
HuT 78 (IC50 = 4.36μM)

Fig. 4

SEQUENCE LISTING

<110> Prakash, Ramesh K.

<120> Conjugates Targeted to the Target Receptors and/or Interleukin-2 Receptors

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 13

Gly Leu Gln His Leu Phe Leu Gly
1 5

<210> 14

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 14

Gly Leu Glu Arg Ile Leu Leu Gly Phe Leu Gly
1 5 10

<210> 15

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 15

Gly Leu Glu His Ile Leu Leu Gly Phe Leu Gly
1 5 10

<210> 16

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 16

Gly Leu Gln His Ile Leu Leu Gly Phe Leu Gly
1 5 10

<210> 17

<211> 11

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 17

Gly Leu Asp His Ile Phe Leu Gly Phe Leu Gly
1 5 10

<210> 18

<211> 11

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 18

Gly Leu Asn His Ile Phe Leu Gly Phe Leu Gly
1 5 10

<210> 19

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 19

Thr Gly Leu Gln His Ile Leu Leu Gly Phe Leu Gly
1 5 10

<210> 20

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 20

Ser Leu Gln His Ile Leu Leu Gly Phe Leu Gly
1 5 10

<210> 21

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Biodegradable spacer sequence

<400> 21

Gly Phe Leu Gly
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<210> 22

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 22

Thr Gly Leu Asn Arg Ile Leu Leu Gly Phe Leu Gly
1 5 10

<210> 23

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 23

Thr Gly Leu Asn Arg Leu Leu Leu Gly Phe Leu Gly
1 5 10

<210> 24

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 24

Thr Gly Leu Asp Arg Ile Leu Leu
1 5

<210> 25

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 25

Thr Gly Leu Asp Arg Leu Leu Leu
1 5

<210> 26

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 26

Thr Gly Leu Asn Arg Leu Leu Leu
1 5

<210> 27

<211> 8

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 27

Thr Gly Leu Asn Arg Ile Leu Leu
1 5

<210> 28

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 28

Thr Gly Leu Asp Arg Ile Phe Leu Gly
1 5

<210> 29

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 29

Thr Gly Leu Asp Arg Leu Phe Leu Gly
1 5

<210> 30

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 30

Thr Gly Leu Asn Arg Ile Phe Leu Gly
1 5

<210> 31

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 31

Thr Gly Leu Asn Arg Leu Phe Leu Gly
1 5

<210> 32

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 32

Gly Leu Asn Arg Ile Leu Leu
1 5

<210> 33

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 33

Leu Asp Arg Ile Leu Leu
1 5

<210> 34

<211> 7

<212> PRT

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 34

Gly Leu Asp Arg Leu Leu Leu
1 5

<210> 35

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 35

Leu Asp Arg Leu Leu Leu
1 5

<210> 36

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 36

Gly Leu Asn Arg Leu Leu Leu
1 5

<210> 37

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 37

Leu Asn Arg Leu Leu Leu
1 5

<210> 38

<211> 7

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 38

Gly Leu Asn Arg Ile Leu Leu
1 5

<210> 39

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 39

Leu Asn Arg Ile Leu Leu
1 5

<210> 40

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 40

Gly Leu Asp Arg Ile Phe Leu Gly
1 5

<210> 41

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 41

Leu Asp Arg Ile Phe Leu Gly
1 5

<210> 42

<211> 8

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 42

Gly Leu Asp Arg Leu Phe Leu Gly
1 5

<210> 43

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 43

Leu Asp Arg Leu Phe Leu Gly
1 5

<210> 44

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 44

Gly Leu Asn Arg Ile Phe Leu Gly
1 5

<210> 45

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 45

Leu Asn Arg Ile Phe Leu Gly
1 5

<210> 46

<211> 8

<212> PRT

<213> Artificial Sequence

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<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 46

Gly Leu Asn Arg Leu Phe Leu Gly
1 5

<210> 47

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Exhibits sequence similarity to a portion of human IL-2.

<400> 47

Leu Asn Arg Leu Phe Leu Gly
1 5